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Papillomatous digital dermatitis spirochetes suppress the bovine macrophage innate immune response

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Abstract

Papillomatous digital dermatitis (PDD) is a polymicrobial infection in soft tissue adjacent to the hoof and is the leading cause of lameness in dairy cattle. *Treponema phagedenis*-like (TPL) spirochetes are a constant feature of PDD lesions and are localized deep in infected tissue. Host–cell response mechanisms to TPL spirochetes are poorly understood. To assess how bovine macrophages respond to cellular constituents of TPL spirochetes, changes in transcription were analyzed using serial analysis of gene expression (SAGE) and real time RT-PCR. This analysis revealed that some proinflammatory cytokines (e.g. GCP-2 and IL-8) are induced in treated macrophages, while receptors and their accessory proteins for IL-1, IL-6 and IL-11 are either down regulated or unchanged. Two genes encoding proteins having negative effects on NFκB, IκB and SIVA-1, are significantly induced in stimulated cells. Several genes associated with the cytoskeleton and antigen presentation are down regulated after exposure to sonicated TPL spirochetes, as are genes associated with wound repair. Combined, these data suggest that the innate immune and wound repair functions of bovine macrophages exposed to TPL cellular constituents are impaired thereby enabling bacteria to resist clearance and induce lesion formation. Use of this in vitro bovine macrophage model should be useful in elucidating host–spirochete interactions and facilitate identification of potential virulence traits.
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Keywords: Treponema; Papillomatous digital dermatitis; SAGE; Real time RT-PCR

1. Introduction

Papillomatous digital dermatitis (PDD) is the leading cause of lameness in dairy cattle in North America and Europe. PDD lesions start as a mild dermatitis at the interface between hard and soft tissue within the interdigital cleft of the hoof (Blowey et al.,

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1994). Left untreated, painful ulcers develop and erode hoof tissue (Read et al., 1992). In addition to animal welfare concerns, PDD causes significant economic losses through weight loss, decreased milk production, premature culling and the expense of treatment (Hernandez et al., 2001).

Although PDD is a polymicrobial infection, anaerobic spirochetes belonging to the genus *Treponema* are consistently found in PDD lesions (Collighan and Woodward, 1997; Read et al., 1992). *Treponema phagedenis*-like (TPL) spirochetes are of particular interest, because these bacteria are localized deep within PDD lesions near the interface with healthy tissue (Moter et al., 1998). Furthermore, cattle with PDD demonstrate specific humoral and cell-mediated immune responses to TPL spirochetes (Trott et al., 2003). Combined, these studies suggest TPL spirochetes are important for development of PDD lesions.

The recurrence of PDD lesions after antibiotic treatment suggests immunity developed to natural infections is not protective. Due to a lack of defined animal models, bacterial factors that affect PDD lesion development and persistence remain unknown. This study was undertaken to characterize how bovine macrophages respond to TPL spirochete cellular constituents in vitro, with a long-term goal of using this model to identify TPL factors that affect host–pathogen interactions. The results of this study suggest TPL spirochetes impair various macrophage functions including innate immunity and wound repair, thereby facilitating PDD lesion development.

2. Materials and methods

2.1. Bacteria and bovine macrophages

T. phagedenis-like strain 1A was isolated in Iowa from a cow with PDD (Trott et al., 2003), and propagated in New Oral Spirochete (NOS) medium supplemented with 10% rabbit serum (Haapasalo et al., 1991) under anaerobic conditions (100% N_2). *Escherichia coli* DH10B (Invitrogen Corp.) was used for transformation of plasmids. *E. coli* was propagated on LB medium containing 50 μg/ml zeocin.

An immortalized bovine macrophage cell line (BoMac) was used throughout this study (Stabel and Stabel, 1995). Cells were propagated in RPMI 1640 medium (GIBCO) containing 10% fetal calf serum (Colorado Serum Company), 100 μg/ml streptomycin and 100 U/ml penicillin (Sigma Chemical Corp.) at 37 °C with 5% CO_2.

2.2. Stimulation of bovine macrophages and RNA isolation

Bacteria in the exponential phase of growth were harvested by centrifugation (10,000 × *g* for 15 min) and washed twice with PBS. Bacteria were lysed by sonication using short-wave pulses of 30 s each (Vibra Cell, Sonics and Materials Inc.) until no intact cells were observed by dark field microscopy. The protein concentrations of sonicated *Treponema* suspensions were determined using the Bradford assay (Bio-Rad Laboratories) following the manufacturers instructions. Sonicated TPL preparations contained less than 0.2 ng DNA per μg protein, as determined by spectrophotometry after proteinase K digestion. Sonicated TPL contained less than 0.05 endotoxin units per μg of protein, as determined by the *Limulus* amebocyte lysate assay (E-Toxate, Sigma Chemical Corp.).

Bovine macrophages grown to about 90% confluence were stimulated with either soncated TPL (1 μg/ml) or *E. coli* O111:B4 LPS (1 μg/ml) suspended in complete RPMI 1040. Cells were incubated at 37 °C for 4 h (sonicate and LPS) and 8 h (sonicate) before RNA extraction. Cell monolayers were disrupted with RLT + lysis solution and total cellular RNA isolated with RNeasy kits (Qiagen) according to the manufacturer's instructions. Where appropriate, cell suspensions were further disrupted by homogenization using Qiashredder columns (Qiagen). Residual DNA was removed by DNaseI treatment, followed by RNA purification (Qiagen).

2.3. Construction and analysis of SAGE libraries

Cellular mRNA was enriched from total RNA using the PolyATract mRNA isolation system II (Promega) and used as starting material for preparation of SAGE libraries. Two SAGE libraries were prepared from 5 μg ds-cDNA, one each from resting and sonicate-treated macrophages (4 h treatment) following established protocols (Velculescu et al., 1995, 1997; Zhang et al., 1997) using NlaIII as the anchoring enzyme. Concatermerized ditags were size

selected by electrophoresis through polyacrylamide gels, then ligated with SphI-digested pZERO-1 (Invitrogen Corp.) and used to transform *E. coli* to zeocin resistance. Transformed colonies were picked at random to 96-well plates and plasmid DNA prepared by alkaline lysis (Qiagen). Plasmid DNA was sequenced unidirectionally from the T7 primer region using fluorescent dideoxy chain-termination reactions separated in a 3700-sequence analyzer (Applied Biosystems Inc.).

2.4. Data processing

Sequence data interpreted using Phred (Ewing and Green, 1998; Ewing et al., 1998) and Sequencher (Gene Codes Corp.) were combined and edited manually. Inserts from a total of 2155 and 2131 plasmids were sequenced from control and stimulated cell libraries, respectively. Data files for each library were processed with eSAGE software (Margulies and Innis, 2000) to extract ditags, remove duplicate sequences, and align tags with the *Bos taurus* Unigene database (Build #79, 15 June 2006) after processing with the ehm-tag-Mapping Perl scripts (Margulies et al., 2001). SAGE tags were also mapped against the *B. taurus* Gene Index v. 12 after processing the database with a Perl script used to identify 10 bp tags downstream from the 3′ proximal NlaIII site. Singlets (tags that appeared only once in the combined libraries) were removed from analysis if they did not correlate to known genes or SAGE tags in existing *B. taurus* data sets (GSM48351, GSM48352, GSM48353, GSM11383, GSM24604, GSM29563, GSM39287, GSM11027, GSM11028, GSM3036 and GSM3037). Differences in tag frequencies were evaluated for a 0.05 level of significance using two tests (Audic and Claverie, 1997; Kal et al., 1999).

2.5. Semiquantitiative RT-PCR

RNA quality was assessed using 2100 Bioanalyzer and RNA 6000 Nano Assay chips (Agilent Technologies Inc.) and RNA concentration was measured on a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc.). First strand cDNA synthesis was performed using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen Corp.) according to the manufacturer's protocol. Two micrograms of total RNA with Oligo(dT)$_{12–18}$ primer was reverse transcribed to single-stranded cDNA per 100 μl reaction. DNase-free RNase H was added to each reaction to remove the RNA template. First-strand cDNAs were purified by extraction using Quick-Clean resin (BD Biosciences) and DNA concentrations determined by spectrophotometry.

Semiquantitative RT-PCR was done in a DNA Engine Opticon® 2 detection system (MJ Research Inc.) using QuantiTect™ SYBR Green PCR Kit (Qiagen), gene specific primers (Supplemental Table 1), with 200 ng/μl cDNA template. PCR primers were designed using Primer Design 5 software (Sci. Ed. Software) from sequences obtained from GenBank. All reactions had at least two technical replicates for each sample collected from independent biological replicates. RT-PCR data were analyzed by using the $2^{-(\Delta\Delta C_T)}$ method (Livak and Schmittgen, 2001) using β-actin as the control gene (ΔC_T) and non-stimulated samples as the calibrator ($\Delta\Delta C_T$).

3. Results

Expression profiles of resting and TPL-sonicate treated bovine macrophages were determined using SAGE, coupled with semi-quantitative RT-PCR. A total of 150,832 SAGE tags were sequenced with 78,550 tags from resting cells and 72,243 tags from cells treated with sonicated TPL. Both libraries lacked GC bias and were of high sequence quality. Combined, these SAGE libraries contained a total of 32,343 unique tags, of which 10,671 were linked to known genes or previously identified, but uncharacterized transcripts. Approximately one-quarter (7549) of the SAGE tags were singlets that lacked identity with tags in either library or available *B. taurus* SAGE databases. Although a portion of these singlets may be the result of sequencing error, high singlet rates may also be due to detection of macrophage-specific transcripts, microRNA precursors, or the result of alternative mRNA splicing and polyadenylation signals.

To gain insight into the expression profile of resting bovine macrophages, the most abundant transcripts in untreated cells were identified (Table 1). Osteonectin (SPARC), a matricellular protein associated with the extracellular matrix (ECM) was the most prevalent transcript in resting macrophages. Ferritin, thymosin beta 4, Ef1α, ribosomal proteins, cytoskeletal proteins (α-tubulin and β-actin), and cytochrome C oxidase

Table 1
Most abundant transcripts in resting bovine macrophages

Protein	SAGE tag	Tag count	Transcript abundance (%)
Osteonectin	CAAACTCTTG	682	0.87
Ribosomal protein P1	GGCTTTGGTC	616	0.78
α-Tubulin	TGTGTCTGTA	584	0.74
Thymosin, β4	TTGGTGAAGG	448	0.57
Cytochrome C oxidase subunit I	CCTCGACGAT	355	0.45
Cytochrome C oxidase subunit II	AGCTGTGCCC	336	0.43
NADH dehydrogenase subunit 1	TGACACGTAT	294	0.37
Actin, cytoplasmic 1	TGAGAACATT	280	0.36
Ef1α	AGACAGACAG	278	0.35
Ferritin heavy chain	CATATTTGGG	277	0.35
Ribosomal protein L21	GCCTGATGGG	267	0.34
Ribosomal protein L10	CTCACCAATA	262	0.33
Intestinal cysteine-rich protein 1	TTTGGACCCA	241	0.31
Insulin-like growth factor binding protein 7	CGTATCATTA	235	0.30
HDL binding protein isoform 2	GTGACCACGG	235	0.30
Translationally controlled tumor protein	TAGGTTGTCT	234	0.30
Ribosomal protein S23	CTGTTGGTGA	228	0.29
Ribosomal protein S3	CCCCAGCCGG	220	0.28
Ribosomal protein S6	GCAGAGTTCG	218	0.28
Ribosomal protein S29	GGTCACCAGC	215	0.27
Ribosomal protein P2	GGCTTCGGCT	214	0.27
Cytochrome oxidase subunit III	TGATTTCACT	213	0.27
Retinoblastoma-binding protein 2	CTGGGAAATT	209	0.27
Collagen, type I, α1	ACCAAAAACC	209	0.27
Transgelin	ACGGGCTATG	208	0.26

Tag count is relative to the 78,550 total SAGE tags sequenced from resting BoMac cells and abundance is the percentage of each SAGE tag relative to the total sample analyzed.

subunits, were also highly transcribed, a finding consistent with a pattern of active growth and iron regulation. The capacity of BoMac cells to respond to LPS was determined by semi-quantitative RT-PCR analysis of RNA isolated from treated and untreated cells. Granulocyte chemotactic factor-2 (GCP-2) and IL-8 were increased in LPS treated cells (Supplementary Table 2), indicating that BoMac cells are capable of a proinflammatory response.

Comparison of tag frequencies in SAGE libraries from resting and TPL sonicate-treated macrophages showed that the level of most of transcripts (>31,000) were unaffected by treatment. Within these two libraries, transcription of 546 genes was significantly altered ($P < 0.05$) by exposure to sonicated TPL; transcription of 255 genes was increased, and transcription of 291 genes was decreased, after treatment. The levels of selected transcripts were confirmed by real time RT-PCR (Table 2) of independently prepared biological replicates.

To assess the effect of TPL cellular material on macrophages, SAGE expression data were examined primarily for changes in genes encoding proteins associated with four major functions; immune modulation, apoptosis, cytoskeletal structure and wound repair, including an analysis of transcriptional regulators and signal proteins related to these functional categories (Table 3). Macrophage exposure to sonicated TPL altered significantly ($P < 0.05$) transcription of several genes that affect NFκB or its function (Table 3). Although NFκB transcription was unchanged, transcription of genes encoding the NFκB inhibitor, IκB and SIVA-1, a protein that negatively regulates NFκB (Gudi et al., 2006), were increased ≥5-fold (Table 3).

Surprisingly few cytokine or chemokine genes were induced by treatment of macrophages with sonicated TPL. Transcription of GCP-2 and IL-8 increased in sonicate-treated cells, and this response was time dependent, increasing over the 8 h time course (Table 2). It should be noted that IL-6 and IL-8

Table 2
Comparison of changes in transcription using SAGE and real time RT-PCR

Gene	Fold-change of transcript		
	SAGE	RT-PCR	
	4 h	4 h (S.D.)	8 h (S.D.)
BCL-2	1	−1.14 (0.06)	1.13 (0.24)
Ferritin H	−1.3	−1.03 (0.09)	−1.04 (0.21)
GCP-2	7	2.10 (0.24)	5.33 (0.68)
GMCSF	ND	−1.16 (0.63)	1.60 (0.16)
IκB	6	1.49 (1.11)	2.00 (0.97)
IL-1β	ND	ND	ND
IL-6	ND	1.33 (0.36)	1.88 (0.59)
IL-8	ND	2.00 (1.34)	6.23 (2.43)
IL-18	−4	−2.22 (0.29)	−1.04 (0.42)
MCP-1	ND	−1.51 (0.57)	−1.03 (0.18)
Osteonectin	−1.4	−1.69 (0.39)	−1.03 (0.14)
p53	−2.8	−1.59 (0.36)	−1.12 (0.18)
TNFα	ND	ND	ND
TWEAK	−2	−1.45 (0.23)	−1.39 (0.10)

Changes in transcription were calculated either by comparing tag frequencies as described for SAGE (Margulies and Innis, 2000) or by the $2^{-(\Delta\Delta C_T)}$ method (Livak and Schmittgen, 2001) for RT-PCR reactions using actin as the control gene and resting cells as the calibrator. Data presented are from three independent experiments: RNA from one biological replicate was used to prepare SAGE libraries, while RNA from two biological replicates were used for RT-PCR analysis to assure independence from material used for SAGE libraries. Changes in transcription levels for SAGE data were calculated after normalizing tag levels to 75,000. Fold-changes in transcript levels for RT-PCR data represent mean and standard deviations (S.D.) from the two independently prepared biological replicates. There were at least two technical RT-PCR replicates for each sample. Transcript signals below the detection level of the method used (>70,000 SAGE tags per library or 40 PCR cycles) is indicated as not detected (ND).

transcription were below the level of detection using SAGE at the level of sequencing used in this study; transcripts for these genes were only detected by RT-PCR (Table 2). No transcription of IL-1β or TNFα was detected in sonicate-treated cells. Several genes typically induced by LPS or lipoproteins, remained unchanged, including GMCSF, IL-6, IL-18 (Table 2), CXCL3 and gelsolin (data not shown).

Several pro- and anti-apoptotic genes displayed changes in transcription as a result of exposure to sonicated TPL. Transcription of three pro-apoptotic genes, *bok*, optineurin, and SIVA-1 increased, while transcription of TWEAK, p53 binding protein and PERP, a potent pro-apoptotic protein that interacts with p53, decreased (Table 3 and data not shown). Sonicate-treated macrophages also increased transcription of the anti-apoptotic gene encoding Bcl-X_l. No evidence of apoptosis in TPL-treated BoMac cells was detected using a flow cytometry-based assay (data not shown).

Exposure of macrophages to TPL-sonicates altered expression of several genes encoding cytoskeletal proteins or proteins associated with antigen presentation functions. Specifically, transcripts encoding actinin alpha 1, cytoskeleton-associated protein 1, cytohesin 1, actinin alpha 3 and connexin 43 (Table 3 and data not shown) decreased and may disrupt formation of tight junctions involved in cell–cell interactions, such as those involved in antigen presentation. Transcription of GTP-binding proteins Rac1, RalB and CDC42 and their effectors (Table 3 and data not shown) were altered by exposure to TPL spirochete sonicates, which may contribute to changes in actin organization in treated cells.

Wound repair functions were also affected by TPL spirochete sonicates; transcription of genes encoding connective tissue growth factor, early growth response protein 1 and genes associated with insulin-like growth factors was decreased in treated macrophages (Table 3 and data not shown). Likewise, genes encoding insulin-like growth factors or their receptors were downregulated in TPL-sonicate treated macrophages (Table 3). Although metalloprotease gene transcription was unaffected by exposure to sonicated TPL, transcription of tissue inhibitor of metalloprotease 2 decreased significantly in treated cells (Table 3).

4. Discussion

The global transcriptional profiles of resting and TPL-activated bovine macrophages were determined using SAGE. SAGE analysis is open-ended, the data can be reanalyzed as genes are identified, and thus the data continue to grow in value as the genome of an organism becomes more thoroughly annotated. Therefore, transcripts can be identified from existing data over time, making this approach particularly well suited for characterizing global expression profiles on animals for which the genome annotations are underdeveloped. BoMac cells are commonly used as a model for studying host–pathogen interactions,

Table 3
Functional categorization of selected genes

Function	SAGE tag	Tag number		Fold-change
		Resting cells	Treated cells	
Immune modulation				
CD40	GATATGCAGT	0	7	>7
Granulocyte chemotactic protein 2 (GCP-2)	TAGTTTATAA	1	7	7
IL-6 signal transducer	TGCAAATCGT	11	2	−5.5
Lymphotoxin beta receptor	GTGCTCAACA	7	0	>−7
T-cell immune regulator 1	CCACACGGTG	3	12	4
TREM-1	TGCTGCAACT	8	1	−8
Apoptosis				
BCL2-related ovarian killer (*bok*)	TGGCAGGGAG	1	12	12
Bcl-X_l	CTTTCTTGCC	0	6	>6
HSPC016	GCGCCTGCCG	1	9	9
Optineurin	TTACGGACTG	1	9	9
PERP apoptosis effector	GAATATTATT	13	4	−3.3
Antigen presentation/cytoskeleton				
Actinin alpha 1	AAAATATTTT	12	3	−4
Cytohesin-1	ACTGAAAGTT	5	0	>−5
Cytoskeleton-associated protein 1	TATTAAATTT	9	1	−9
Cytoskeleton-associated protein 4	AAAACCTCGG	0	4	>4
MHC class I heavy chain	TCTATCCCTG	38	94	2.5
Antigen H13	TATCGGCTTT	11	2	−5.5
Glypican 1	CCAAGGGCCT	37	18	−2.1
Wound healing				
Connective tissue growth factor (CTGF)	TTTGCACCTT	182	64	−2.8
Insulin-like growth factor binding protein 7 (IGFBP-7)	CGTATCATTA	224	93	−2.4
Insulin-like growth factor II (IGF-II)	AACCCCTCAA	9	1	−9
Tissue inhibitor of metalloproteinase 2 (TIMP-2)	AATAAAAACA	32	12	−2.7
Transcriptional regulation/cell signaling				
Cbp/p300-interacting transactivator	AAAAGATACT	8	0	>−8
CDC42 effector protein 5	GACGCGGAGC	3	16	5.3
Interferon regulatory factor 3 (IRF-3)	GTCAAAAAAA	1	7	7
NFκB inhibitor (IκB)	GAGCCCCAGA	1	6	6
Rac1	GCCAAGGAGA	4	17	4.3
RalB	TGAAATATAA	15	4	−3.8
SIVA-1	GGAGGAAGGC	0	5	>5

SAGE tag frequencies were normalized for 75,000 per library, and these values were used to calculate the magnitude of change (fold-change) between resting and stimulated cells. Positive values indicate that transcription increased, while negative values indicate that transcription was decreased in response to treatment with sonicated TPL spirochetes. Differences in transcript levels for all genes shown were significant ($P < 0.05$) as determined according to the method described by Audic and Claverie (1997).

and their availability and consistency provide advantages over use of primary cells. However, established cell lines, such as BoMac cells, often lack features common to primary cells. For example, primary macrophages and BoMac cells differ in supporting intracellular growth of *Mycobacterium avium* subsp. *paratuberculosis* (Woo et al., 2006), and BoMac cells lack several surface markers common to primary macrophages, including CD11b or CD14 (Sager et al., 1999). This latter finding was supported by transcript analysis; SAGE tags for CD11b or CD14 were not detected in the combined libraries. Nonetheless, the most abundant transcripts in resting BoMac cells also comprise some of the most abundant transcripts in primary human monocytes and monocyte-derived dendritic cells, including ferritin, thymosin β4, Ef1α, ribosomal proteins and cytochrome C oxidase subunits (Hashimoto et al., 1999; Suzuki et al., 2000). In

addition, BoMac cells increased transcription of GCP-2 and IL-8 after treatment with *E. coli* LPS indicating a capacity to mount a proinflammatory response. Lack of detectable endotoxin in the sonicated TPL implies that the transcriptional responses detected in treated cells are specific to exposure to TPL spirochete cellular constituents. Consequently, BoMac cells provide a useful model to study macrophage–TPL spirochete interactions.

The results of this study suggest TPL spirochetes that reside deep within PDD lesions have a net immunosuppressive effect on bovine macrophages and may disrupt wound repair. This finding is consistent with histological analysis of PDD lesions that provided evidence of an ineffective macrophage response to infection (Blowey et al., 1994). Reduced transcription of several cytoskeletal protein genes (Table 3) may impair communication between antigen presenting cells and lymphocytes thereby blocking development of an acquired immune response sufficient for bacterial clearing. In addition, transcription of several cytokine genes was altered in macrophages exposed to sonicated TPL. Although IL-8 and GCP-2 transcription were increased, transcription of several proinflammatory cytokines was either unchanged or reduced in macrophages exposed to sonicated TPL. Coinciding with a reduction in inflammatory cytokine transcription, sonicate treated macrophages may be more responsive to IL-10; transcription of the IL-10 receptor was increased in treated cells. IL-10 inhibits Th1 cytokine production thereby helping to restrict the immune response, and IL-10 was recently shown to interfere with clearance of *Borrelia burgdorferi* in a mouse model (Lazarus et al., 2006).

Transcription of genes regulated by NFκB is likely altered as a result of exposure to sonicated TPL. Although NFκB transcription is unchanged, transcription of the NFκB activating protein gene is suppressed and transcription of two negative regulators of NFκB, IκB and SIVA-1, is increased. Reduced transcription of the gene encoding CREP binding protein (Cbp) p300-interacting transactivator, which is needed by interferon regulatory factor 3 (IRF-3) for functional activity may also impair the ability of macrophages to clear spirochetes from PDD lesions. Although IRF-3 transcription was increased in response to exposure to sonicated TPL, reduction of Cbp likely impairs its functional activity. Furthermore, Cbp also interacts with p53 during transcriptional gene activation (Livengood et al., 2002), and evidence for the combined effect of decreased transcription of Cbp and p53BP1 is seen in the reduced or unchanged transcription of several p53-regulated genes including glypican 1 (Table 3) and ephrin A4 precursor (data not shown). Reduced expression of PERP, and unchanged levels of Bax, Bak and Bcl-2, combined with increased expression of Bcl-X_l are consistent with an anti-apoptotic response to TPL sonicates; macrophages treated with sonicated TPL do not undergo apoptosis.

TPL spirochetes may impair the ability of cattle to repair damage at the PDD lesion during infection as suggested by decreased transcription of CTGF, EGR-1, IBP-7 and IGF-II. Increased MMP activity is common in chronic wounds (Wall et al., 2002), and we expect PDD lesions should have increased matrix metalloprotease activity leading to increased degradation of collagen through the reduced expression of TIMP-2 (Woessner, 1991). These findings suggest that TPL spirochetes promote lesion propagation by enhancing tissue degradation via endogenous protease activity combined with impaired expression of tissue growth factors.

5. Conclusion

The findings of this study suggest that cellular constituents from TPL spirochetes impair macrophage function, and may limit their ability to clear bacteria from PDD lesions. Host cell responses to pathogenic spirochetes vary greatly, and this may be partly due to inherent properties of the bacteria. For example, *Treponema denticola* utilize a surface protease, dentilysin, to degrade IL-1β, IL-6 and TNFα (Miyamoto et al., 2006), which may alter host cytokine induction; *T. denticola* induces substantially less IL-6, IL-8 or MCP-1 in gingival fibroblasts than *Treponema pectinovorum* (Nixon et al., 2000). Exposure of bovine macrophages to sonicated TPL spirochetes alters transcription of several genes that influence the ability of these cells to promote both innate and acquired immune responses by the host. These activities, combined with disruption of wound repair, likely contribute to persistence of PDD lesions. Identification of the bacterial components responsible

for these activities should facilitate improved methods for disease prevention and control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vetmic.2007.06.001.

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